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Applying the Airbrakes: Treating Mitochondrial Disease with Hypoxia

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A paper from Jain et al. (2016) using whole-genome CRISPR knockout libraries in human cells and models of mitochondrial disease suggests chronic hypoxia could be an unexpected treatment for disorders of mitochondrial respiration.

Mitochondria are responsible for the production of 90% of cellular ATP during oxidative phosphorylation (OXPHOS), a process that couples cellular respiration to ATP production. Specifically, electrons are transferred from NADH or FADH2 via multi-subunit respiratory chain complexes and carriers to molecular oxygen, with the concomitant proton electrochemical gradient being harnessed by a fifth complex, the FoF1 ATP synthase, to form ATP. Defects in the mitochondrial respiratory chain (RC) underlie many inherited mitochondrial disorders and have been implicated in multiple neurological and metabolic diseases. The development of treatments for mitochondrial dysfunction has been the focus of intensive research across multiple labs; however, effective treatments have proved to be elusive. Several strategies for treatment development are currently being followed (Lightowlers et al., 2015). The majority of these involve increasing the production of ATP by the RC, either by providing more substrate for OXPHOS in the form of NADH precursors (Khan et al., 2014), increasing mitochondrial biogenesis to add to the pool of mitochondria available for ATP production (Viscomi et al., 2011), targeting dysfunctional mitochondria for degradation via mitophagy (Villanueva Paz et al., 2016), or degrading mitochondrial genomes harboring disease-causing mutations (Bacman et al., 2013; Gammage et al., 2014) and thus improving mitochondrial health. Recent work by Jain and colleagues in the Mootha laboratory has now highlighted a new paradigm in treating mitochondrial diseases (Jain et al., 2016).

Using a large-scale CRISPR Cas9-mediated whole-genome knockout screen, the authors were able to identify a set of genes whose ablation improved survival in cells with pharmacologically impaired RC complex III. Surprisingly, the most effective genetic suppressor of mitochondrial disease was inhibition of Von Hippel-Lindau (VHL) factor, a key regulator in the hypoxia response pathway and recognized tumor suppressor protein. Under physiologically normoxic conditions, a group of hypoxia-inducible transcription factors (HIFs) are produced and are hydroxylated, signaling them for ubiquitination and subsequent degradation by the VCB-Cull2 complex. VHL, a core component of this complex, is the E3 ubiquitin ligase. Under hypoxic conditions, this hydroxylation step does not occur, thus preventing degradation of HIFs and allowing cells to respond in environments with low oxygen tension. The authors also show that VHL knockout cells are resistant to other inhibitors of OXPHOS (RC complex I and FoF1 ATP-synthase). Further, by using a small-molecule inhibitor (FG-4592) of the HIF hydroxylating enzyme prolyl-hydroxylase, Jain et al. rescued a growth defect in cell lines with Complex I, III, or V inhibition.

These are intriguing observations, so could hypoxia, counter-intuitively, help whole organisms that suffer from an RC defect? Strikingly, it appears so. Jain et al. demonstrate that a mildly hypoxic environment (11% O_2) improves lifespan in the *Ndufs4* mouse model of Leigh's syndrome, the rare and fatal pediatric mitochondrial disease that is simulated in mice by the loss of a component of RC complex I. Under normoxic (21% O_2) conditions, these mice die after ~60 days. Under hypoxic conditions, HIF was stabilized in the mice and caused transcription of hypoxic factors including erythropoietin, a well-established marker for hypoxia. Several indicators of disease progression, such as maintenance of body weight and temperature, were improved, along with a dramatic restoration of their locomotor function. Conversely, survival was severely impaired by hyperoxic conditions (55% O_2), with mice dying after a few days of exposure to this condition.

The authors postulate that several mechanisms may be acting to improve the lifespan of the mice. First, the reduction in O_2 tension could be decreasing the production of reactive oxygen species (ROS), which have been implicated in the pathogenesis of mitochondrial dysfunction (Hayashi and Cortopassi, 2015). Second, hypoxia may be activating HIFs, which will lessen the production of ATP by OXPHOS via a reduced supply of carbon to the TCA cycle and increase the reliance on glycolysis (Kim et al., 2006) (Figure 1). Indeed, the authors show that several key glycolytic enzymes are increased in response to hypoxia. It is interesting to note that work from Johnson et al. (Johnson et al., 2013) appears to contradict this finding. Although their study showed that treatment of a similar *Ndufs4* KO model with tacrolimus, the mTOR inhibitor, also improved the phenotype, it was suggested that this was due to metabolic remodeling leading to a decrease in glycolytic intermediates. These two contrasting findings will need further investigation; however, it could indicate that the reduction in ROS

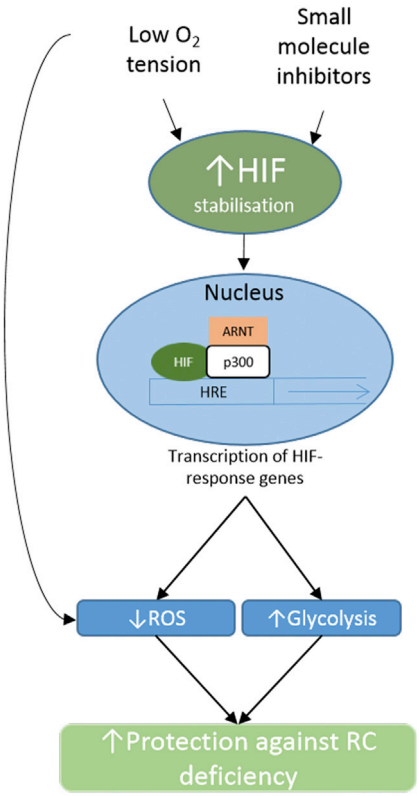

Figure 1. Proposed Mechanism of Hypoxic Treatment for Mitochondrial Disease
Stabilization of HIF, either by decreased oxygen tension or small-molecule inhibitors of HIF degradation, enables HIF translocation to the nucleus. Stabilized HIF, in conjunction with its β subunit ARNT and transcriptional coactivator p300, begins a program of gene transcription causing increased glycolysis, decreased OXPHOS, and, consequently, a decline in ROS production, which is also directly lowered by low O_2 tension. These factors combined may be protective against RC deficiency in mitochondrial disease.

during hypoxia could be the key factor in the improvements described, rather than the promotion of glycolytic ATP production.

As Jain et al. point out, prior to use as a treatment, the relationship between ROS availability, mitochondrial respiration, and the physiological changes at the organ level need to be deciphered. Although the authors used FG-4592 to induce the hypoxia response in cell lines and zebrafish models, the high concentration (50 μM) and the impermeability to the blood-brain barrier means that other small molecules will have to be developed to replicate hypoxia in the central nervous system, the primary system affected by mitochondrial disease. One safety issue that may also need to be explored if considering treatment is that, although the triggering of the hypoxia response appears to ameliorate the RC disorders, stability of certain HIFs can be tumorigenic, consistent with the role of VHL as a tumor suppressor.

Although healthy humans can acclimatize to living in low-oxygen environments such as the high Andes, the ability of mitochondrial disease patients to adapt to these conditions is unknown. This might be particularly true for patients who already have symptoms rather than those who adapt before symptoms develop. Patients with Leigh's syndrome are often hypoxic due to hypoventilation or infection, and whilst hyperoxia is harmful in the *Ndufs4* mice, careful monitoring of oxygen tension in patients is crucial during acute relapses. As the authors state, further work is needed to understand the effect of hypoxic conditions between 11% and 20% O_2 and intermittent hypoxic treatment, as these may be better tolerated. Clearly there are many issues that need to be addressed; however, this work is exciting and could open up a new and unexpected front in the battle against mitochondrial disease.

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